

**Increasing the value of mushrooms as functional foods: induction of alpha and beta glucan content via novel cultivation methods**

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**Final Report for BARD-4851****Final Abstract**

During the granting period, we performed the following projects:

- 1) Firstly, we differentially measured glucan content in several pleurotus mushroom strains. Mushroom polysaccharides are edible polymers that have numerous reported biological functions; the most common effects are attributed to  $\beta$ -glucans. In recent years, it became apparent that the less abundant  $\alpha$ -glucans also possess potent effects in various health conditions. In our first study, we explored several *Pleurotus* species for their total,  $\beta$  and  $\alpha$ -glucan content. *Pleurotus eryngii* was found to have the highest total glucan concentrations and the highest  $\alpha$ -glucans proportion. We also found that the stalks (stipe) of the fruit body contained higher glucan content than the caps (pileus). Since mushrooms respond markedly to changes in environmental and growth conditions, we developed cultivation methods aiming to increase the levels of  $\alpha$  and  $\beta$ -glucans. Using olive mill solid waste (OMSW) from three-phase olive mills in the cultivation substrate. We were able to enrich the levels mainly of  $\alpha$ -glucans. Maximal total glucan concentrations were enhanced up to twice when the growth substrate contained 80% of OMSW compared to no OMSW. Taking together this study demonstrate that *Pleurotus eryngii* can serve as a potential rich source of glucans for nutritional and medicinal applications and that glucan content in mushroom fruiting bodies can be further enriched by applying OMSW into the cultivation substrate.
- 2) We then compared the immune-modulating activity of glucans extracted from *P. ostreatus* and *P. eryngii* on phagocytosis of peripheral blood neutrophils, and

superoxide release from HL-60 cells. The results suggest that the anti-inflammatory properties of these glucans are partially mediated through modulation of neutrophil effector functions (*P. eryngii* was more effective). Additionally, both glucans dose-dependently competed for the anti-Dectin-1 and anti-CR3 antibody binding. We then tested the putative anti-inflammatory effects of the extracted glucans in inflammatory bowel disease (IBD) using the dextran sulfate sodium (DSS)–induced model in mice. The clinical symptoms of IBD were efficiently relieved by the treatment with two different doses of the glucan from both fungi. Glucan fractions, from either *P. ostreatus* or *P. eryngii*, markedly prevented TNF- $\alpha$  mediated inflammation in the DSS–induced inflamed intestine. These results suggest that there are variations in glucan preparations from different fungi in their anti-inflammatory ability.

- 3) In our next study, we tested the effect of glucans on lipopolysaccharide (LPS)-induced production of TNF- $\alpha$ . We demonstrated that glucan extracts are more effective than mill mushroom preparations. Additionally, the effectiveness of stalk-derived glucans were slightly more pronounced than of caps. Cap and stalk glucans from mill or isolated glucan competed dose-dependently with anti-Dectin- and anti-CR-3 antibodies, indicating that they contain  $\beta$ -glucans recognized by these receptors. Using the dextran sulfate sodium (DSS)-inflammatory bowel disease mice model, intestinal inflammatory response to the mill preparations was measured and compared to extracted glucan fractions from caps and stalks. We found that mill and glucan extracts were very effective in downregulating IFN- $\gamma$  and MIP-2 levels and that stalk-derived preparations were more effective than

from caps. The tested glucans were equally effective in regulating the number of CD14/CD16 monocytes and upregulating the levels of fecal-released IgA to almost normal levels. In conclusion, the most effective glucans in ameliorating some IBD-inflammatory associated symptoms induced by DSS treatment in mice were glucan extracts prepared from the stalk of *P. eryngii*. These spatial distinctions may be helpful in selecting more effective specific anti-inflammatory mushrooms-derived glucans.

- 4) We additionally tested the effect of glucans on lipopolysaccharide-induced production of TNF- $\alpha$ , which demonstrated stalk-derived glucans were more effective than of caps-derived glucans. Isolated glucans competed with anti-Dectin-1 and anti-CR3 antibodies, indicating that they contain  $\beta$ -glucans recognized by these receptors. In conclusion, the most effective glucans in ameliorating IBD-associated symptoms induced by DSS treatment in mice were glucan extracts prepared from the stalk of *P. eryngii* grown at higher concentrations of OMSW. We conclude that these stress-induced growing conditions may be helpful in selecting more effective glucans derived from edible mushrooms.
- 5) Based on the findings that we could enhance glucan content in *Pleurotus eryngii* following cultivation of the mushrooms on a substrate containing different concentrations of olive mill solid waste (OMSW) and that these changes are directly related to the content of OMSW in the growing substrate we tested the extracted glucans in several models. Using dextran sulfate sodium (DSS)–inflammatory bowel disease (IBD) mice model, we measured the colonic inflammatory response to the different glucan preparations. We found that the histology damaging score

(HDS) resulting from DSS treatment reach a value of  $11.8 \pm 2.3$  were efficiently downregulated by treatment with the fungal extracted glucans, glucans extracted from stalks cultivated at 20% OMSW downregulated to a HDS value of  $6.4 \pm 0.5$  and at 80% OMSW showed the strongest effects ( $5.5 \pm 0.6$ ). Similar downregulatory effects were obtained for expression of various intestinal cytokines. All tested glucans were equally effective in regulating the number of CD14/CD16 monocytes from  $18.2 \pm 2.7$  % for DSS to  $6.4 \pm 2.0$  for DSS +glucans extracted from stalks cultivated at 50% OMSW.

- 6) We finally tested glucans extracted from *Pleurotus eryngii* grown on a substrate containing increasing concentrations of olive mill solid waste (OMSW) contain greater glucan concentrations as a function of OMSW content. Treatment of rat Intestinal epithelial cells (IEC-6) transiently transfected with Nf- $\kappa$ B fused to luciferase demonstrated that glucans extracted from *P. eryngii* stalks grown on 80% OMSW downregulated TNF- $\alpha$  activation. Glucans from mushrooms grown on 80% OMSW exerted the most significant reducing activity of nitric oxide production in lipopolysaccharide (LPS) treated J774A.1 murine macrophages. The isolated glucans were tested *in vivo* using the Dextran Sodium Sulfate (DSS) induced colitis in C57Bl/6 mice and found to reduce the histology damaging score resulting from DSS treatment. Expression of various intestinal cytokines were efficiently downregulated by treatment with the fungal extracted glucans. We conclude that the stress-induced growing conditions exerted by OMSW induces production of more effective anti-inflammatory glucans in *P. eryngii* stalks.

**Describe how the contribution of the collaboration between the laboratories contributed to the research.**

Our research is based on the unique contribution of the three teams involved:

- 1) ISRAELI PARTNER: Dr. Ofer Danay whose expertise in novel growing conditions of edible mushrooms.
- 2) ISRAELI PARTNERS: Prof. Betty Schwartz, Prof. Yitzhak Hadar: Experts in nutrition, biochemistry of mushrooms, cell biology allowed test the inherent changes in the different mushroom *Pleurotus* species following stress conditions during growth.
- 3) AMERICAN PARTNER: Prof. Vaclav Vetvicka: Tested in a wide variety of *in vivo* and *in vitro* systems all different mushroom *Pleurotus* species grown under different conditions and primarily tested by the ISRAELI PARTNERS: Prof. Betty Schwartz, Prof. Yitzhak Hadar.

Dr. Ofer Danaï was in charged of obtaining the mushrooms crops. He and his group tested strains and conditions to obtain mushrooms containing high concentrations of alpha and beta glucans under controlled conditions but as similar as possible to real farm conditions. He distributed to Prof. Schwartz and Hadar laboratories for quantitative analyses of the mushrooms content of glucan. Prof. Schwartz and Hadar group conducted a wide variety of *in vitro* assays to test the anti-inflammatory nature of the mushroom glucans isolates in transfected intestinal cells. The elected mushroom glucans isolates were then forwarded to Prof. Vetvicka by FedEx in order to test their anti-inflammatory activities *in vivo*.

Interestingly, the best mushrooms-glucans preparations (O.D.) where those containing the highest glucans concentrations, they exerted the most remarkable *in vitro* anti-inflammatory effects (B.S. and Y.H.) and concomitantly were demonstrated to exert the most anti- effective anti-inflammatory effects in *in vivo* settings (V.V).

The exchange of samples and the complimentary expertise of the partners ensured synergistic effort, fruitful cooperation and allowed accruing hitherto a

significant mass of results that enabled the groups to submit a large number of manuscripts for publication.

## **Final Report for BARD-4851**

### **The major achievements accomplished in the project to date**

- 1) We compared the immune-modulating activity of glucans extracted from *P. ostreatus* and *P. eryngii* on phagocytosis of peripheral blood neutrophils, and superoxide release from HL-60 cells. The results suggest that the anti-inflammatory properties of these glucans are partially mediated through modulation of neutrophil effector functions (*P. eryngii* was more effective). Additionally, both glucans dose-dependently competed for the anti-Dectin-1 and anti-CR3 antibody binding. We then tested the putative anti-inflammatory effects of the extracted glucans in inflammatory bowel disease (IBD) using the dextran sulfate sodium (DSS)–induced model in mice. The clinical symptoms of IBD were efficiently relieved by the treatment with two different doses of the glucan from both fungi. Glucan fractions, from either *P. ostreatus* or *P. eryngii*, markedly prevented TNF- $\alpha$  mediated inflammation in the DSS–induced inflamed intestine. These results suggest that there are variations in glucan preparations from different fungi in their anti-inflammatory ability.
- 2) *Pleurotus eryngii* is recognized for its prominent nutritional and medicinal value. In our study, we tested the effect of glucans on lipopolysaccharide (LPS)-induced production of TNF- $\alpha$ . We demonstrated that glucan extracts are more effective than mill mushroom preparations. Additionally, the effectiveness of stalk-derived glucans were slightly more pronounced than of caps. Cap and stalk glucans from mill or isolated glucan competed dose-dependently with anti-Dectin- and anti-CR-3 antibodies, indicating that they contain  $\beta$ -glucans recognized by these receptors. Using the dextran sulfate sodium (DSS)-inflammatory bowel disease mice model, intestinal inflammatory response to the mill preparations was measured and compared to extracted glucan fractions from caps and stalks. We found that mill and glucan extracts were very effective in downregulating IFN- $\gamma$  and MIP-2 levels and that stalk-derived preparations were more effective than from caps. The tested glucans were equally effective in regulating the number of CD14/CD16 monocytes and upregulating the levels of fecal-released IgA to almost normal levels. In conclusion, the most effective glucans in ameliorating some IBD-inflammatory associated symptoms induced by DSS treatment in mice were glucan extracts prepared from the stalk of *P. eryngii*. These spatial distinctions may be helpful in selecting more effective specific anti-inflammatory mushrooms-derived glucans.
- 3) Using dextran sulfate sodium (DSS)–inflammatory bowel disease (IBD) mice model, we measured the colonic inflammatory response to the different glucan preparations. We found that the histology damaging score (HDS)



resulting from DSS treatment reach a value of  $11.8 \pm 2.3$  were efficiently downregulated by treatment with the fungal extracted glucans, glucans extracted from stalks cultivated at 20% OMSW downregulated to a HDS value of  $6.4 \pm 0.5$  and at 80% OMSW showed the strongest effects ( $5.5 \pm 0.6$ ). Similar downregulatory effects were obtained for expression of various intestinal cytokines. All tested glucans were equally effective in regulating the number of CD14/CD16 monocytes from  $18.2 \pm 2.7$  % for DSS to  $6.4 \pm 2.0$  for DSS +glucans extracted from stalks cultivated at 50% OMSW.

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- 4) We treated rat Intestinal epithelial cells (IEC-6) transiently transfected with Nf- $\kappa$ B fused to luciferase demonstrated that glucans extracted from *P. eryngii* stalks grown on 80% OMSW downregulated TNF- $\alpha$  activation. Glucans from mushrooms grown on 80% OMSW exerted the most significant reducing activity of nitric oxide production in lipopolysaccharide (LPS) treated J774A.1 murine macrophages. The isolated glucans were tested in vivo using the Dextran Sodium Sulfate (DSS) induced colitis in C57Bl/6 mice and found to reduce the histology damaging score resulting from DSS treatment. Expression of various intestinal cytokines were efficiently downregulated by treatment with the fungal extracted glucans. We conclude that the stress-induced growing conditions exerted by OMSW induces production of more effective anti-inflammatory glucans in *P. eryngii* stalks.

## Publications for Project IS-4851-15 R

S t a t u s	T y p e	Authors	Title	Journal	V o l u m e : P a g e s	Y e a r	C o u n t r y
Publi shed	Revie wed	Avni S, Ezov N, Hanani H, Yadid I, Karpovsky M, Hayby H, Gover O, Hadar Y, Schwartz B, Danay O	Olive mill waste enhances $\beta$ -Glucan content in the edible mushroom Pleurotus eryngii	<i>Int J Mol Sci</i>	18 (7) : E1 56 4	<b>20 17</b>	IS onl y
Publi shed	Revie wed	Vaclav Vetvicka, Ofer Gover, Hilla Hayby, Ofer Danay , Nirit Ezov , Yitzhak Hadar and Betty Schwartz	Spatial Distribution of Glucan Type and Content between Caps and Stalks in Pleurotus eryngii: Impact on the Anti- inflammatory Functionality	<i>Int J Mol Sci</i>	19( 11) : pii: E3 37 1	<b>20 18</b>	Joi nt
Acce pted	Revie wed	Vaclav Vetvicka, Ofer Gover, Hilla Hayby, Ofer Danay , Nirit Ezov , Yitzhak Hadar and Betty Schwartz	Immune- Modulating Activities of Glucans Extracted from Pleurotus ostreatus and Pleurotus eryngii	<i>J of Func Foods</i>	In pre ss :	<b>20 19</b>	Joi nt
Subm itted	Revie wed	Vaclav Vetvicka, Ofer Gover, Hilla Hayby, Ofer Danay ,	Immunomodulating Effects Exerted by Glucans Extracted from the Mushroom Pleurotus eryngii Grown in Substrates	<i>Int J Medicinal Mushrooms</i>	:	<b>20 19</b>	Joi nt

		<i>Nirit Ezov , Yitzhak Hadar and Betty Schwartz</i>	Containing Various Concentrations of Olive Mill Waste				
Subm itted	Revie wed	<i>Ofer Gover, Hilla Hayby, Adi Levy, Elena Fishman, Ofer Danay, Nirit Ezov, Yitzhak Hadar, Vaclav Vetvicka, Betty Schwartz</i>	Enhanced Anti- Inflammatory Effects by Glucans Extracted from the Stalks of Pleurotus eryngii Grown in Substrates Containing Olive Mill Waste	<i>J of Func Foods</i>	:	<b>20 19</b>	Joi nt